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Effects of lipid peroxidation and antioxidant status on peak flow in a population aged 59–71 years: the EVA study

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KEYWORDS

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 α -Tocopherol;
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Lung function

Summary Oxidative stress is implicated in age-related diseases and is a possible determinant in the loss of lung function. The aim of our study was to examine the association between blood indicators of oxidative metabolism and lung function in an old population. The relationships of three antioxidant indicators (selenium, total carotenoids and α -tocopherol) and of a marker of lipid peroxidation (thiobarbituric acid-reactive substances (TBARs)) with age and height-adjusted PEF were assessed in 688 subjects aged 59–71 years (61% never smokers, 30% ex-smokers, 9% current smokers). Stratified analyses according to gender and smoking were performed. Gender, age, tobacco and alcohol consumption, educational level and body mass index were taken into account as potential confounders. Regarding antioxidant markers, PEF was significantly positively associated with total carotenoids in the whole group ($P = 0.03$), and with selenium among ex-smokers only ($P = 0.008$). Regarding lipid peroxidation, PEF was significantly negatively associated with TBARs in men only ($P = 0.02$). Consistent results were observed when analyzing quantitative values and quartiles of biological markers. Results are consistent with the hypothesis of the role of both oxidants and antioxidants on lung function in elderly. Research is needed to better understand the effect of former smoking in the surviving elderly subjects.

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Introduction

Increasing evidence supports the role of oxidative stress in ageing, cardiovascular and respiratory diseases. Oxidant exposures originate from smoking, other environmental factors and systemic

oxidants released during inflammatory process. Inadequate antioxidant defense in particular from diet may result in oxidative stress. Studies based on dietary intake and based on serum levels of nutrients provide complementary information.¹ Whereas studies based on serum and dietary intake are consistent regarding the protective role of vitamin C on lung function, results concerning vitamin E are less established.² Only recently carotenoids have been studied separately from retinol.² There is no definite evidence on the role of total or specific carotenoids on lung function and

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there is limited information in elderly subjects, an age range insufficiently studied.³ Lycopene, alpha and beta carotene were found to be associated in a Dutch elderly study,⁴ whereas beta cryptoxanthin and lutein/zeaxanthin were associated to lung function in a large study conducted in an American general population, consistent with a recent report on the same population showing that dietary intake of carotenoids other than beta carotene may be involved.⁵ The presence of Se in the active site of GSH-Px is essential to its function—that is, removing hydrogen peroxide damaging lipid and phospholipid hydroperoxides generated in vivo by reactive oxygen species and thus to the antioxidant status of the cell and results from the NHANES study showed an association of serum selenium with FEV₁, stronger for current smokers than non- and ex-smokers.¹ Lipid peroxidation of membrane lipids is one of the direct effect of oxidant exposure. Epidemiological evidence supports the hypothesis that Se concentration reflects its use against reactive oxygen species generated by oxidant exposure such as tobacco smoke or coal dust.⁶ Measuring directly oxidative stress is difficult. Assessing lipid peroxidation products, through thiobarbituric acid reactant substances, provides one marker. It has already been shown that TBARs is related to early phases of atherosclerosis, assessed by carotid plaques,⁷ poor cognitive functioning⁸ and cognitive decline⁹ in an ongoing longitudinal study focused on arterial ageing (EVA, étude du vieillissement artériel) conducted in the general population. It has been recently prospectively shown that PEF, which is known to be good predictor of overall mortality in elderly^{10,11} was a predictor, among other variables,¹² of the development of carotid atherosclerotic plaques¹² in the EVA study. In one study conducted in non- and ex-smokers, lung function was negatively associated with TBARs.¹³

The objective of the present study is to assess the relationships of PEF at baseline in the elderly subjects of the EVA study with lipid peroxidation marker (TBARs), and various serum markers of antioxidants (red blood cell alpha tocopherol, plasma selenium and total carotenoids).

Subjects and methods

Subjects

As part of a longitudinal study of vascular and cognitive aging (EVA study), peak flow measurements were recorded at baseline in 788 volunteers

aged 59–71 years recruited from the electoral rolls of Nantes (a city in the west of France) and, to a lesser extent, via information campaigns. Whenever a subject was recruited, her or his spouse was always asked to participate in the study if he or she was in the correct age range. Details on subjects who volunteered to participate in the EVA study have been reported previously.¹⁴ Out of the whole population of the EVA study ($n = 1389$), which examination started in July 1991, peak flow measurements were introduced in June 1992, that is for the second year of enrollment of the subjects. The study protocol was approved by the Comité d'éthique de l'hôpital du Kremlin Bicêtre and an informed written consent was obtained from all participants.

Examination of the subjects

Data on demographic background, educational achievement, past and present medical history, cigarette smoking and alcohol consumption were collected during a baseline face-to-face interview. Three levels of educational achievement were considered: junior high school (6–9 years) or less, senior high school (10–12 years), university (> 12 years). According to their smoking behavior at the time of examination, subjects were classified as life-long never smokers, ex-smokers or current smokers. For ever smoker, life-time amount of smoking was defined by pack-years being equal to smoking 20 cigarettes a day for 1 year. Three levels of alcohol consumption were determined: teetotalers, drinkers with low (≤ 20 g of alcohol/day), and high consumption (> 20 g of alcohol/day). Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m²). Peak flow measurements were performed with the same apparatus (Mini Wright peak flow meter) for all subjects as previously described.¹² Three technically acceptable maneuvers were recorded and the maximum value used in analysis. Adequate lung function measures were obtained for 93% of the subjects. Subjects with and without lung function were similar for all biological indicators under study. Finally, 688 subjects without asthma were included in the analysis.

Biological indicators

Three biological indicators of anti-oxidant status (plasma selenium, plasma total carotenoids and red blood cell α -tocopherol) and one marker of lipoperoxidation (TBARs) were measured as previously described.¹⁴ Plasma levels of TBARs

were determined by fluoremetric method as described by Richard et al.¹⁵ Plasma carotenoids were measured by spectrophotometry after precipitation of plasma proteins with ethanol. Red blood cell vitamin E (α -tocopherol) was assessed by HPLC and results expressed in μmol per gram of hemoglobin or μmol per liter unit Plasma selenium was determined by electrothermal atomic absorption spectrometry (EEAS).

Statistical analysis

All analyses were performed on PEF z-score based on the regression of PEF on age and height in each gender. Due to their skewed distributions, plasma carotenoids level and plasma TBARs level were log transformed. Quartile values of biological indicators were also used in analyses. Statistical analyses included correlation coefficients, analysis of variance, multiple linear regression and covariance analyses. In multivariate analyses, models included age (in years), tobacco status (never smokers, ex-smokers with moderate cumulative tobacco consumption (≤ 20 pack-years), ex-smokers with heavy consumption (> 20 pack-years), current smokers), alcohol intake (teetotalers, drinkers with low or high consumption), education level (junior high school or less, senior high school, university), and BMI (kg/m^2). Interactions were formally tested for smoking and for gender. All analyses were carried out using the Statistical Analysis System (SAS) version 6.12.

Results

Analysis has been performed in 416 women and 272 men, aged 65.2 ± 3.0 years (Table 1). Most women (84%) had never smoked and most men (59%) were ex-smokers. Few subjects (9%) were still smoking at the time of examination. PEF z-score was lower in smokers than in ex-smokers and in never smokers (-0.49 ± 1.0 , 0.02 ± 1.0 , 0.06 ± 0.97 , respectively $P < 0.001$). PEF was lower in subjects with a BMI lower than $20 \text{ kg}/\text{m}^2$ ($n = 40$, $\beta = -0.4$; $P = 0.007$), but no relation was found with a BMI higher than 30 ($n = 77$). PEF was not significantly related to educational achievement, alcohol consumption.

Plasmatic carotenoid level was higher in women than in men and related negatively to BMI ($r = -0.40$; $P = 0.0001$) and positively to educational achievement. After adjustment for gender, carotenoid level was not related to smoking habits but was lower in alcohol drinkers than in teetotalers with a difference of borderline significance

($P = 0.08$), and selenium level was related to smoking ($P = 0.03$) with higher values for non- and ex-smokers than current smokers. Plasmatic level of α -tocopherol did not relate to any of the parameters studied. TBARs were positively related to alcohol consumption ($P = 0.002$). Biological markers were moderately correlated with each other: carotenoids were positively correlated with selenium ($r = 0.11$; $P = 0.007$) and α -tocopherol ($r = 0.13$; $P = 0.002$), TBARs were negatively correlated with α -tocopherol ($r = -0.11$; $P = 0.005$).

Lipid peroxidation

Plasma TBARs was not correlated to PEF on the whole sample. This relation remained unchanged when adjustment for smoking, alcohol, education and BMI (Table 2). Considering TBARs quartiles, some trend was apparent with a PEF decrease from the lowest to the highest quartiles of plasma TBARs, but without reaching statistical significance (Fig. 1). Analyses stratified according to gender showed a negative association in men ($n = 264$, $r = -0.14$; $P = 0.02$) but not in women ($n = 395$, $r = -0.02$; $P = 0.75$). Smoking and lipid level did not modify the relation of TBARs with PEF.

Antioxidant makers

Plasma carotenoid level was positively correlated to PEF ($n = 660$, $r = 0.08$; $P = 0.03$). Red blood cell α -tocopherol and selenium level were not correlated to PEF. Regression coefficients for PEF against biological indicators before and after adjustment for confounding factors are shown in Table 2. After adjustment for possible confounding factors, the regression coefficients remained significant for plasma carotenoids ($\beta = 0.187$, $\text{SD} = 0.085$, $P = 0.03$).

There was no significant interaction between tobacco status and carotenoids, α -tocopherol, in relation to PEF, but there was an interaction of smoking with selenium level ($P = 0.05$). In ex-smokers plasma selenium level was correlated to PEF ($n = 203$, $r = 0.17$; $P = 0.02$), while there were no association in smokers ($n = 59$, $r = -0.12$; $P = 0.4$) or in never smokers ($n = 396$, $r = -0.02$; $P = 0.6$). After adjustment, the relation of plasma selenium to PEF remained statistically significant in ex-smokers ($\beta = 0.951$, $\text{SD} = 0.353$, $P = 0.008$).

Analyses of PEF among quartiles of biological indicators confirmed the linear relations. Subjects belonging to the highest quartiles of plasma carotenoids had higher PEF than those belonging to the lowest quartiles, with an increase in PEF of

Table 1 Main characteristics of the study population.

	Men (<i>n</i> = 272)	Women (<i>n</i> = 416)	All (<i>n</i> = 688)
Age (years) (mean ± SD)	65.1 ± 3.2	65.2 ± 2.9	65.2 ± 3.0
Educational level			
Junior school or less, %	45.6	51.1	48.9
High school, %	33.5	37.8	36.1
University, %	20.9	11.1	15.0
Alcohol intake			
Teetotalers, %	14.8	41.4	30.9
≤ 20 g/day, %	38.4	48.7	44.6
> 20g/day, %	46.8	9.9	24.5
Tobacco status			
Never smokers, %	26.1	84.2	61.2
Ex-smokers ≤ 20 pack-years, %	28.3	7.9	16.0
Ex-smokers > 20 pack-years, %	30.5	3.1	13.9
Current smokers, %	15.1	4.8	8.9
Body mass index (kg/m ²) (mean ± SD)	26.8 ± 3.2	24.7 ± 4.1	25.5 ± 3.9
Peak flow (L/min) (mean ± SD)	506 ± 80	365 ± 55	421 ± 96
Plasma TBARS ^a (μmol/L) (mean ± SD)	2.92 ± 0.35	2.97 ± 0.40	2.95 ± 0.38
Plasma carotenoids (μmol/L) (mean ± SD)	2.30 ± 1.05	3.12 ± 1.30	2.79 ± 1.27
Red blood cell α-tocopherol (μmol/L) (mean ± SD)	5.57 ± 1.22	5.61 ± 1.19	5.59 ± 1.2
Plasma selenium (μmol/L) (mean ± SD)	1.06 ± 0.20	1.09 ± 0.19	1.07 ± 0.20

^aTBARS = thiobarbituric acid-reactive substances.

Table 2 Regression of PEF on lipid peroxidation marker and antioxidants in the whole sample.

	<i>n</i>	<i>b</i> ^a	(SE)	<i>P</i>	<i>n</i>	Adjusted <i>b</i> ^b	(SE)	<i>P</i>
Plasma TBARS ^c level (μmol/L)	659	−0.497	(0.307)	0.11	656	−0.465	(0.303)	0.12
Plasma total carotenoids level (μmol/L)	660	0.167	(0.078)	0.03	657	0.187	(0.085)	0.03
Red blood cell α-tocopherol (μmol/L)	624	−0.011	(0.033)	0.75	621	−0.017	(0.033)	0.60
Plasma selenium level (μmol/L)	658	0.184	(0.196)	0.35	655	0.197	(0.196)	0.31

All models were run on PEF z-scores.

^aRegression coefficient without adjustment.

^bRegression coefficient with adjustment for tobacco status, alcohol consumption, educational level and body mass index.

^cTBARS = thiobarbituric acid-reactive substances.

14 L/min from the lowest to the highest quartile. No relation was observed with red blood cell α-tocopherol. Table 3 shows mean PEF rate according to selenium level quartiles, stratified on tobacco status. In the largest group of ex-smokers (*n* = 202), after adjustment for pack-years, alcohol consumption, BMI, and educational level, PEF z-score were 0.448, −0.023, 0.137, −0.258 from the highest to the lowest quartile of plasma selenium, *P* = 0.003. No trend was observed in never smokers or smokers.

Combined analysis of lipid peroxidation and antioxidant markers

The relations of PEF with TBARS and total carotenoids remained unchanged when introducing the four biological indicators (quartiles) in the same multivariate regression model. After exclusion of subjects with a history of pulmonary or cardiovascular diseases (*n* = 64), the association TBARS with PEF became of borderline significance in the whole sample, but the other associations remained

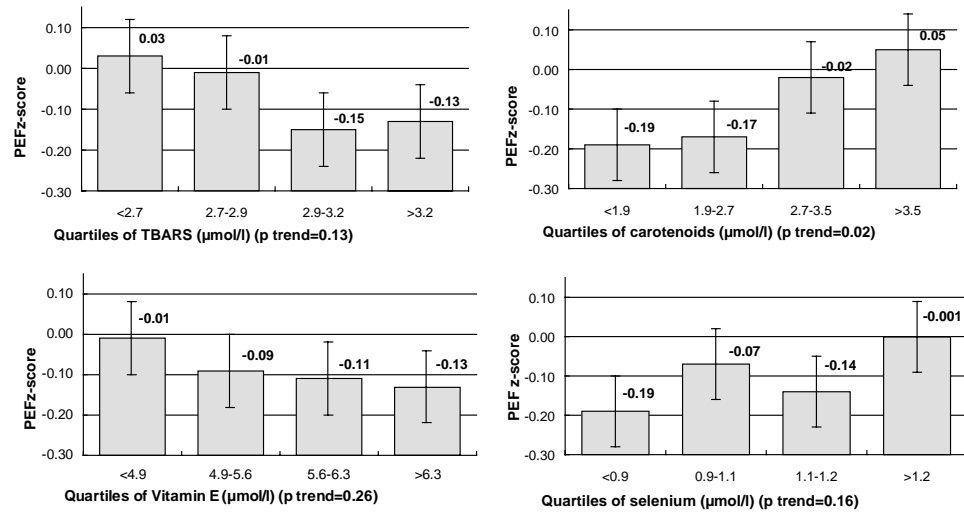


Figure 1 PEF z-scores according to quartiles of lipid peroxidation and antioxidant markers after adjustment for tobacco status, alcohol consumption, educational level, and Body Mass Index.

Table 3 Adjusted^a PEF according to selenium levels quartiles and to tobacco status.

	Non-smokers			Ex-smokers			Smokers		
	<i>n</i>	Mean (SE)	<i>p</i> trend	<i>n</i>	Mean (SE)	<i>p</i> trend	<i>n</i>	Mean (SE)	<i>p</i> trend
Plasma selenium level ($\mu\text{mol/L}$)									
Quartile I (<0.94)	94	0.121 (0.11)	0.82	43	-0.258 (0.16)	0.003	17	-0.140 (0.30)	0.18
Quartile II (≥ 0.94 –<1.07)	105	0.217 (0.11)		54	0.137 (0.15)		16	-0.493 (0.28)	
Quartile III (≥ 1.07 –<1.20)	101	0.154 (0.12)		47	-0.023 (0.16)		8	-0.124 (0.41)	
Quartile IV (≥ 1.20)	95	0.178 (0.12)		58	0.448 (0.15)		17	-0.837 (0.31)	

^aAll models including alcohol consumption, educational level and body mass index were run on PEF z-scores.

unchanged. Further analysis conducted in subjects in the lowest quartile of at least one antioxidant marker confirmed the decrease in PEF according to TBARS quartiles of borderline significance ($n = 363$, mean residual PEF = 0.10; 0.0; -0.09; -0.13; $P = 0.08$).

Discussion

In a French ageing population, PEF was significantly positively related to plasma total carotenoids, one antioxidant and, in men only, negatively to TBARS, a marker of lipid peroxidation, indirect indicator of oxidant status. In ex-smokers, plasma selenium was positively related to PEF. There was no association of red blood cell α -tocopherol with PEF.

Strengths of the study were the large population-based sample of elderly volunteers and the availability of both markers of antioxidant status and of oxidative stress, by the assessment of TBARS. Results from the EVA study have already shown

expected values for the biological markers studied with plasma selenium levels in the same range as that of most European populations, associations of the biological indicators studied with expected risk factors¹⁴ as well as with cardiovascular and cognitive outcomes.^{7–9} One limitation was the lack of dietary data which provides complementary information to serum markers, but few epidemiological studies have included both.¹ Furthermore, it has been shown that plasma indicators, especially carotenoids, were more correlated to actual levels of antioxidant in pulmonary tissue than dietary intake measured by questionnaire.¹⁶ Two studies showed a good correlation between bronchoalveolar lavage or pulmonary tissue and plasma level of carotenoids.^{16,17} The correlation of plasma and pulmonary selenium levels is unclear but some authors suggested a good correlation¹⁸ Other limitations relate to the lack of detailed information of carotenoids, the lack of specificity of TBARS as a marker of lipid peroxidation and of detailed lung function assessment, including FEV₁. These limitations added statistical noise to the exposure

response that could have prevented us from finding effects. Since we did not measure vitamin C, we can not completely avoid a spurious association due to the correlation of vitamin C and other antioxidants. Due to the high occurrence of comorbidities in elderly, detailed assessment of each organ disorder is not possible, which explains why only PEF was available in the EVA study. A limitation of our study was the lack of spirometry in addition to the measure of peak flow. However, even that simple assessment of lung function already allowed to study comorbid conditions and to raise interesting hypotheses regarding the role of lung function in the occurrence of carotid plaques.¹²

Regarding antioxidant markers, results confirm and extend previous observations.^{1,4,5,19,20} In the EVA study, associations with carotenoids were consistent in both genders and not modified when considering other factors associated with its level (BMI, plasma lipid levels, age). No significant interaction was detected with tobacco status. Dietary intake of vitamin A or β -carotene was positively correlated to ventilatory function in two previous studies^{19,20} and not in another one.²¹ Grievink et al.⁴ evidenced a positive trend between three of six components of plasma total carotenoids (α -carotene, β -carotene, lycopene) and FEV₁ in an old population. For Schünemann⁵ the strongest association between FEV₁ and components of total carotenoids was detected for β -cryptoxanthin. Our measurement on the total carotenoids precludes us from detecting a possible relation with one more specific component of the total carotenoids. We did not confirm the association of α -tocopherol with lung function already reported in some studies,^{1,5} but no relation was reported in four other studies with dietary or plasma α -tocopherol.^{4,19,22,23} The choice of red blood cell instead of plasma for evaluating α -tocopherol can also explain the lack of association in our study.

In the large NHANES III study¹ conducted in a general population sample of United States, significant associations of selenium level, which plays a key role in glutathione peroxidase activity, with lung function were reported with stronger associations in current smokers compared to non-smokers and ex-smokers. There were few current smokers in the EVA study. It is interesting to note that the association was observed in ex-smokers, but not in never smokers, supporting the hypothesis of a modifier effect of smoking habits on the relation of selenium level with lung function. The protective effect of selenium could be more important in smokers and ex-smokers who were exposed to a greater amount of exogenous oxidative particles.²⁴ The power of our study may have been insufficient

for finding an effect in the small group of 61 smokers. Another possible explanation for failing to find this association in smokers is that our population for smokers, aged 59–71 years may result from a selection of “healthier” survival smokers. Although the age range was large, no stratification according to age was performed in the large NHANES study. Selection factors in ageing subjects may originate from environmental adaptations, such as quitting smoking, changing dietary habits, but also in relation to gene environment interactions,²⁵ which may be particularly influential for ubiquitous pathophysiological mechanisms such as the oxidant/antioxidant balance.

There have been limited data on the relations of lung function with markers of oxidative stress,¹³ likely because of the uncertainty of the best marker of oxidative damage to use in large scale epidemiologic studies as recently underlined.²⁶ The only study which has assessed the relation of TBARs with lung function was conducted in 132 subjects, aged 37–70 years, non-smokers and ex-smokers.¹³ The authors found a significant negative correlation of TBARs with FEV₁, with similar correlations in men and women. In the present study, the association of TBARs with lung function was significant only in men. This gender difference may be a chance finding, but it is also possible that it has a biological basis. As already reported in the EVA study,²⁷ TBARs were higher in women than in men. Using more specific markers of lipid peroxidation (malondialdehyde and F2-isoprostanes), it has been recently confirmed in healthy adults aged 19–78 years, that lipid peroxidation is higher in women than in men.²⁶ In the present study, differences in lipid level or in alcohol consumption according to gender did not explain that gender difference regarding the association of TBARs with lung function. As higher TBARs have already been reported in postmenopausal than in premenopausal women,²⁸ it suggests that factors associated with oxidative stress may be different according to gender in our study, including men and postmenopausal women, which may explain gender-different associations with lung function.

In most studies subjects had a large range of ages from adulthood to elderly people, only three studies considered specifically older people.^{4,23,29} Dow²⁹ found a positive correlation between dietary α -tocopherol and lung function but not with dietary vitamin C in 188 subjects aged 70–96 years. On the contrary, Morabia²³ found a negative association of the prevalence of obstructive airway with plas-matic retinol level and not with plasmatic carotenoids level neither with α -tocopherol in subjects aged 50–79 years, but the sample of 83 men was

small. In 528 subjects aged 65–85 years, Grievink⁴ evidenced a positive association between lung function and three components of plasma total carotenoids but no relation was found with α -tocopherol. Thus data upon relations between oxidative metabolism and ventilatory function in elderly people are sparse and heterogeneous. Prospective studies in ageing subjects with detailed assessment of oxidant/antioxidant balance through biological markers, assessment of current and cumulative environmental exposure to oxidants (smoking, occupation, ...) and antioxidants (diet) with detailed lung function testing and assessment of comorbidities are warranted.

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